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IMMUNOHISTOCHEMICAL APPROACHES TO DIAGNOSIS IN GYNECOLOGIC PATHOLOGY

Jay H. Beckstead, MD

Immunohistochemical techniques have become widely used in many areas of surgical pathology in recent years. These procedures can provide practical diagnostic information in many areas including gynecologic pathology. The following discussion is organized into sections by major anatomic areas of gynecologic interest. Within each section, specific pathologic questions approachable by immunohistochemical studies are discussed. The scope of this review is limited to relatively practical questions that may be encountered by a practicing pathologist and to commercially available antibodies applicable to paraffin-embedded tissue sections. Given the above parameters, a description of the reagents that may be applicable to the question is presented. This is not intended to imply that all of the reagents need to be applied in each case. Often a single, carefully selected pair of antibodies is completely sufficient to answer the pathologic question. In general, the use of antibodies in pairs serves as an important control in diagnostic interpretation.

VULVA

Paget's Disease Versus Melanoma

This is a relatively straightforward diagnostic problem by immunohistochemical techniques. The malignant epithelial cells of Paget's dis-

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ease express the keratins of simple epithelium (Ker 7, 8, 18, 19).[52] Because the normal vulvar epithelium is similar to the skin expressing Ker 1, 5, 10, and 14,[53, 54] staining with an antibody to low-molecular weight keratins such as CAM 5.2 (Ker 8, 18, 19) will reveal the Paget's cells in stark contrast to the surrounding negative epidermis (Fig. 1). The same cells will often be positive with antibodies to tumor-associated proteins, including carcinoembryonic antigen (CEA) and the tumor-associated glycoprotein-72 (B72.3), and negative with the antigens commonly expressed by melanomas, S-100 protein, and the melanoma-specific antigen HMB-45.[59] Melanomas typically are positive with both S-100 and HMB-45 but lack cytokeratin. However, some melanomas may show reactions with low-molecular weight keratins.[49]

Classification of Vulvar Intraepithelial Neoplasia

Classification of vulvar intraepithelial neoplasia (VIN) is based primarily on morphologic examination, although there are changes in the patterns of keratin expression in this tissue with dysplasia as determined by immunohistochemistry that may be a useful adjunct to diagnosis. The typical keratins expressed in the vulva are 1, 5, 10, and 14[53, 54]; however, these shift dramatically in VIN.[21] Staining with the antibody AE1 (Ker 10, 13, 14, and 19) is confined to the basal layer of the normal

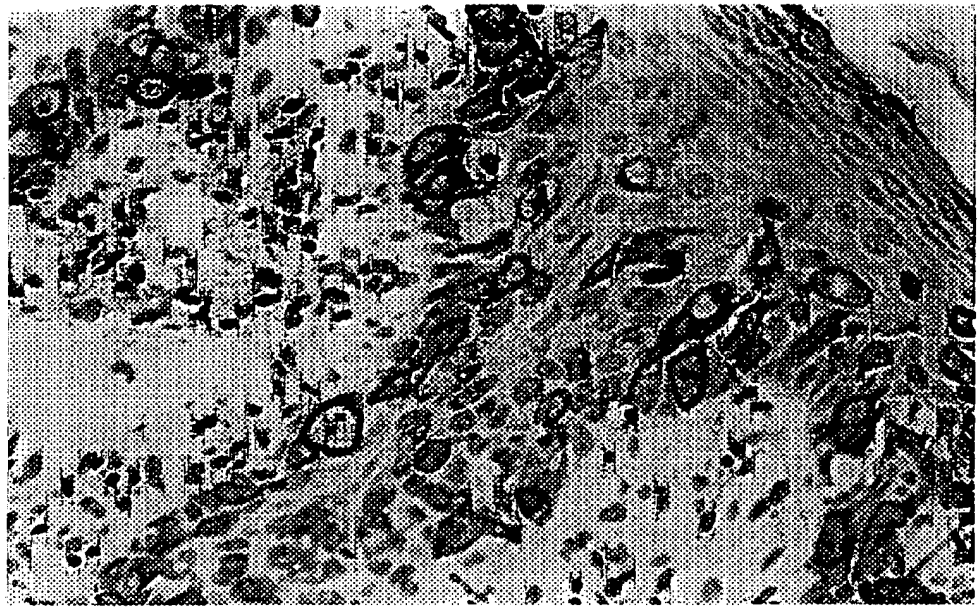


Figure 1. A biopsy of the vulva showing Paget's disease. The malignant cells are strongly labeled by the antibody CAM 5.2, which stains low molecular weight keratins. The surrounding epidermis is negative.

vulvar epithelium; however, in VIN 1 and 2, AE1 staining becomes expressed in the mature layers of epithelium. In VIN 3, AE1 is expressed throughout the epithelium. Warty and basaloid VIN 3 show some differences in the expression of keratins that may be useful in confirming the separation of these two lesions. Warty VIN 3 shows a patchy distribution of AE2 (Ker 1, 2, and 10), whereas basaloid VIN 3 is negative. The low molecular weight keratins, detected by CAM 5.2, are expressed focally in basaloid VIN 3, but not in other forms of VIN or in squamous carcinomas of the vulva.

Separation of Verrucous and Well-Differentiated Squamous Carcinoma

Verrucous carcinomas of the vulva rarely metastasize and should be differentiated from well-differentiated squamous carcinomas at this site. The pattern of keratin distribution with the broad spectrum keratin cocktail AE1/AE3 (Ker 1–8, 10, 13–16, 19) can provide additional data that may be useful in confirming the initial morphologic impression, particularly in biopsy material. Verrucous carcinomas typically show a uniform distribution of AE1/3 throughout the lesion, whereas squamous carcinomas show a patchy distribution of these keratins.[7]

Prognosis in Squamous Carcinoma of the Vulva

Although the use of specific antigenic determinants as prognostic and therapeutic markers is a relatively recent phenomenon, it is likely that its use will increase as clinicopathologic correlations are established. Expression of keratin 10 may have prognostic value in squamous carcinoma of the vulva. Ivanyi and colleagues[32] found that squamous carcinomas expressing keratin 10 did not recur; lesions that did not express the antigen frequently recurred.

CERVIX

Cervical Intraepithelial Neoplasia and Squamous Carcinoma

The grading of cervical intraepithelial neoplasia (CIN) and its separation from invasive squamous carcinoma can often be difficult. Immunohistochemistry can provide some additional data that may sometimes be valuable. CIN shows a keratin pattern similar to that of immature squamous metaplasia with expression of keratins 5, 13, 14, and 17.[27, 33, 70–72] The keratins labeled by CAM 5.2 (Ker 8, 18, 19) are thus usually absent from CIN 1 and 2, and are very infrequently detected in CIN 3; however, they are strongly expressed by invasive squamous CA (Fig.

2).[64] Many different keratins have been reported with invasive cervical squamous carcinomas.[54, 71] However, some authors have noted distinctions between keratinizing carcinomas that usually express keratins 4, 10, and 17 while the same proteins are very infrequently present in nonkeratinizing carcinomas.[33]

There have been several studies that have suggested some diagnostic or prognostic use for CEA in squamous lesions of the cervix, although these remain somewhat controversial. CEA may be positive in squamous metaplasia and CIN I, although the normal squamous cells of the cervix are negative. One study found that the presence of keratin 8 and CEA was associated with a more aggressive clinical course.[16] CIN 3 and invasive squamous cell carcinoma are usually positive with CEA (Fig. 3),[46] but CEA positively has not proven to be valuable in predicting progression of CIN.[43] Estrogen (ER) and progesterone receptors (PR) (PR more frequently) may be expressed in squamous carcinomas of the cervix, but appear to have no prognostic or therapeutic significance.[17, 38, 44, 57, 69] Receptor positivity, however, may be useful in confirming a cervical origin for a squamous carcinoma.

Identification of Early Invasion

Although morphologic recognition of invasion is generally sufficient for diagnosis, immunohistochemistry can provide supportive informa-

Figure 2. A biopsy of the cervix showing normal cervical epithelium overlying an invasive squamous carcinoma. The malignant cells are labeled strongly by the antibody CAM 5.2. The normal mucosa shows only basal layer staining.

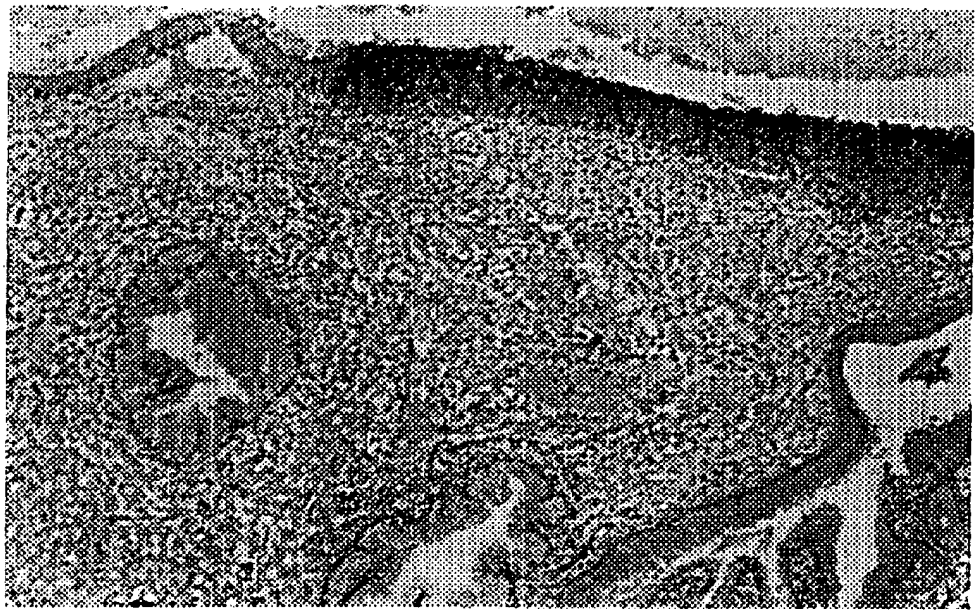


Figure 3. A cervical biopsy showing CIN III. The abnormal squamous cells are strongly positive with antibody to CEA, whereas the adjacent endocervical cells are negative.

tion in difficult cases. Any broad-spectrum keratin can aid in the identification of infiltrating tumor cells and, as noted above, there is a consistent switch to the expression of keratins 8 and 18 with invasion. Another change is the induction of smooth muscle actin in the stromal cells at the site of invasion.[11] Changes in the basement membrane (laminin and collagen type IV)[20] have not proven to be of significant practical value in the diagnosis of early invasion because of the ability of many invasive tumors to produce basement membrane.

ENDOCERVIX

Diagnosis of Endocervical Adenocarcinoma

Adenocarcinomas of the endocervix show the typical keratin profile of a simple epithelium (Ker 7, 8, 18, 19).[54] Small amounts of keratins 4, 14, and 17 have also been observed.[33, 54, 71] These lesions do not express keratins 5 and 6, which are commonly expressed in squamous carcinomas of the cervix. These phenotypic differences may be useful in separating poorly differentiated adenocarcinomas from squamous carcinomas.

Endocervical adenocarcinomas often can be difficult to separate from endometrial adenocarcinomas by morphologic criteria, particularly in biopsy specimens. There are, however, some immunohistochemical marker differences that may assist in this differential diagnosis. Tumors

of endocervical origin commonly are vimentin-negative and CEA-positive (Fig. 4*A*), while those of endometrial origin are vimentin-positive and CEA-negative (Fig. 4*B*).[15, 56]

ENDOMETRIUM

Diagnosis of Endometrial Adenocarcinoma

As noted above, carcinomas of endometrial origin commonly coexpress vimentin and keratins,[48, 63] typically keratins 7, 8, 18, and 19[13, 54] and lesser amounts of the stratification-related keratins 5, 6, 10, 11, 13, 14, 16, and 17.[56] Stratification-related proteins are rarely expressed in adenocarcinomas of the gastrointestinal (GI) tract, kidney, or breast. This may be a useful differential consideration when tumors from these sources must be ruled out. The presence of keratin 7 can also be of some differential importance because it is seldom present in tumors of the lower GI tract.[65] Scattered cells positive with antibodies against glial fibrillary acidic protein (GFAP) have been reported in both endometrial and ovarian adenocarcinomas.[56] Because expression of these proteins is extremely rare outside the nervous system, this may serve as a clue to a gynecologic origin.

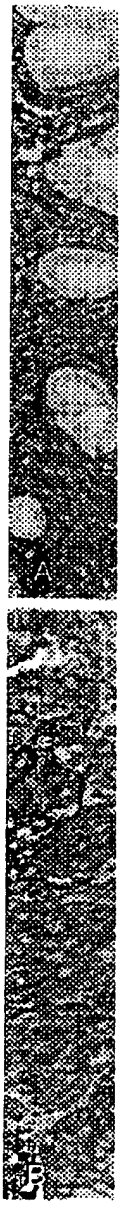
Prognosis in Endometrial Carcinoma

ER and PR status, particularly PR, demonstrates a good correlation with level of differentiation, prognosis, and response to hormonal therapy in endometrial adenocarcinomas,[5, 9, 10, 26, 36] but the use of these data has not been widespread. Discordant data from metastatic and primary sites suggest that multiple sites should be tested for effective results.[68]

Diagnosis of Endometrial Stromal Sarcoma

Endometrial stromal sarcomas may occasionally be difficult to separate from anaplastic carcinomas by morphologic criteria alone. Immunohistochemistry may be helpful in this differential. These mesenchymal tumors typically show strong vimentin positivity and very little keratin, in contrast to carcinomas,[40] although it is clear that small amounts of low-molecular weight keratins may be expressed by scattered tumor cells in these malignancies.[22, 25] Although both the normal endometrial stroma and stromal sarcomas express markers of muscle differentiation (muscle specific actin, smooth muscle actin, desmin), staining is usually focal.[25]

Low-grade endometrial stromal sarcomas often express the ER, but this is rare in high-grade tumors.[60, 75] The presence of ERs has some value in predicting a response to therapeutic hormonal manipulation.



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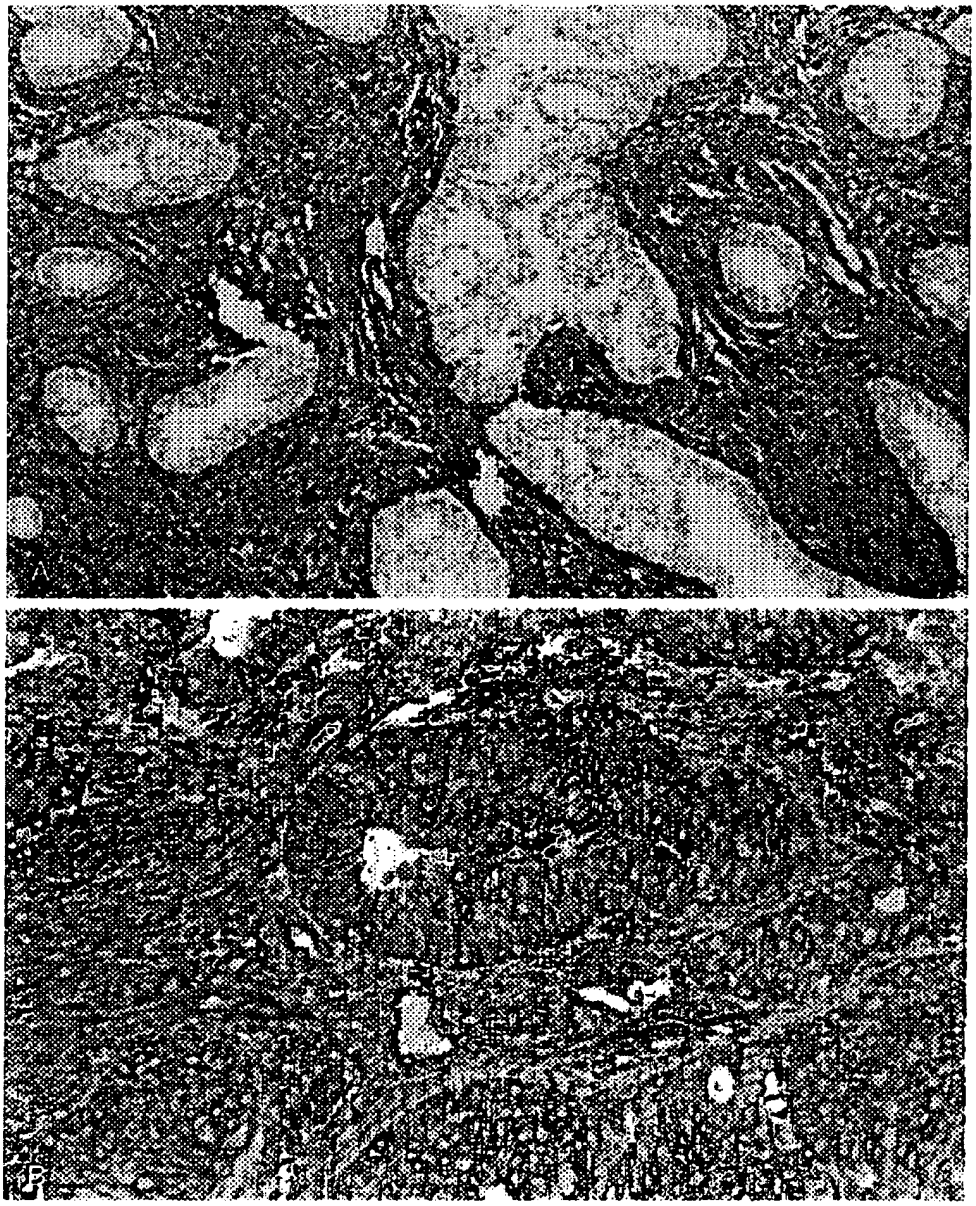

Figure 4. *A*, Section from a moderately differentiated endocervical adenocarcinoma stained with an antibody to vimentin. The adenocarcinoma is completely negative whereas the adjacent stroma show strong labeling. Compare this result with the same marker applied to an endometrial adenocarcinoma in *B*, which shows a section from a moderately differentiated endometrial adenocarcinoma stained with an antibody to vimentin. Both the adenocarcinoma and the adjacent stroma show strong labeling.

Diagnosis of Mixed Mesodermal Tumors

Malignant mixed mesodermal tumors are complex tumors that may show a variety of differentiation pathways. Although these have been traditionally approached morphologically, immunohistochemistry may aid in recognition of the specific mesenchymal elements such as leiomyosarcoma or rhabdomyosarcoma in these tumors.[2, 4, 63, 66, 67]

UTERUS

Diagnosis of Smooth Muscle Tumors

Analysis of intermediate filament proteins is rarely necessary in the diagnosis of smooth muscle tumors in the uterus. In some cases, confirmation of smooth muscle origin with antibodies to desmin or smooth muscle actin may be useful.[3] Importantly, occasional cells in these neoplasms may express low-molecular weight keratins.[8, 28, 62]

FALLOPIAN TUBE AND OVARY

Diagnosis and Classification of Ovarian Adenocarcinoma

Tumors derived from the surface epithelium of the ovary generally express simple epithelial keratins (keratin 7, 8, and 19).[54, 58, 63, 78] Endometrioid carcinomas differ from serous tumors in their expression of keratins 4, 5, and 13,[56] an indication of the potential for squamous differentiation. Most ovarian adenocarcinomas coexpress vimentin with keratin.[48, 56, 63] The major exceptions to this rule are mucinous tumors and Brenner tumors.[78]

Serous ovarian tumors are among the relatively small group of epithelial malignancies that may express the S-100 protein (tumors of breast and salivary gland origin are the other common ones).[42] Although CEA is often positive in mucinous tumors and may be seen in serous, endometrioid, and clear cell tumors, the patchy focal nature of the staining contrasts with the strong diffuse staining typical of gastrointestinal carcinomas.[23, 29]

Small amounts of alpha-fetoprotein (AFP) are relatively common in ovarian embryonal carcinomas and endodermal sinus tumors. This positivity usually appears as scattered cells or clusters of cells. This may be of help in separating endodermal sinus tumors from clear cell adenocarcinomas, which generally lack AFP. In addition, clear cell carcinomas often express the hematopoietic marker CD15, which is rare in yolk sac tumors.[80]

Ovarian hepatoid carcinomas are AFP-positive.[31] AFP is also occasionally reported in other tumors of gynecologic origin.[37, 45]

Separation of Gynecologic Adenocarcinomas from Other Adenocarcinomas

Although separation of adenocarcinomas of other sites from tumors of gynecologic origin may be of significant clinical value, it is often impossible on morphologic grounds. Immunohistochemical reactions can provide significant help in this regard. Adenocarcinomas of the endometrium and ovary are negative with cytokeratin 20, with the exception of some mucinous carcinomas of the ovary. This keratin is commonly expressed in adenocarcinomas of colonic, pancreatic, gastric, and biliary tract origin.[55] Thus, positivity with this marker suggests that a gynecologic primary is much less likely. Another useful marker is HAM56, a macrophage marker that stains many adenocarcinomas except those arising in the digestive tract.[24, 79] Most ovarian and endometrial carcinomas are positive (Fig. 5), which serves as a useful differential point. Staining with keratin 7 is typical of ovarian adenocarcinoma, but is very uncommon in the GI tract.[65]

Placental alkaline phosphatase (PLAP) is often noted in gynecologic carcinomas, including tumors of cervical, endocervical, endometrial (Fig. 6), and ovarian origin.[18] However, it is not commonly expressed by other tumors, with the exception of those of germ cell origin.

CA-125 is an oncofetal antigen commonly expressed in tumors of gynecologic origin, particularly those of ovarian, endometrial, and

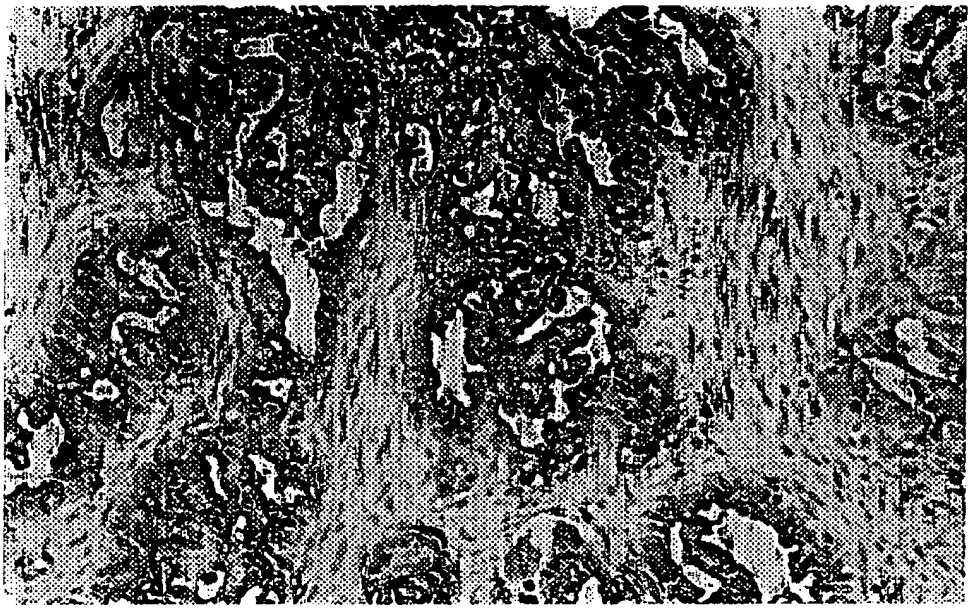

Figure 5. A section from a moderately differentiated endometrial adenocarcinoma stained with the antibody HAM 56. The adenocarcinoma shows strong labeling on the apical surface of the malignant glands.

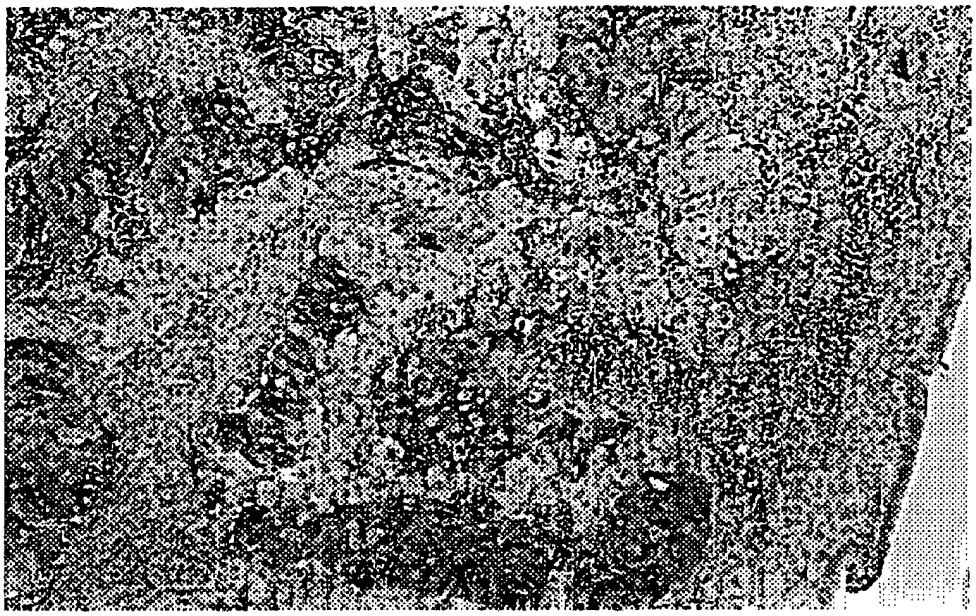

Figure 6. A section from a poorly differentiated ovarian adenocarcinoma stained with antibody to PLAP. The adenocarcinoma shows patchy but definite labeling on the malignant glands.

endocervical origin. It is most frequently positive in serous tumors of the ovary (Fig. 7). Unfortunately, it can also be expressed by adenocarcinomas of other origins. In addition, it can be expressed by hyperplastic cells,[61] further limiting its diagnostic usefulness. In patients whose serum CA-125 levels were not assessed in a timely manner, demonstration of the protein immunohistochemically in a tumor may be important to determine the value of serum levels in follow-up.

B72.3 is a monoclonal antibody to a tumor associated glycoprotein, although it is occasionally expressed in benign cells.[74] Adenocarcinomas of endocervical, endometrial, and ovarian origin are commonly positive with this marker as are adenocarcinomas of the GI tract and lung. B72.3 has been particularly useful in the evaluation of peritoneal cytology specimens because it is negative in mesothelial cells.[73]

The presence of nuclear staining with the estrogen receptor is a strong predictor of gynecologic or breast origin because the only other tumors commonly positive for this marker are those of the thyroid.[19]

Sex Cord-Stromal Tumors

The transitional cell nests of Brenner tumor express keratins 10 and 11[41] and are negative with vimetin.[78] Brenner tumors are the only sex cord-stromal tumors that often express CEA.[34] Granulosa cell tumors are

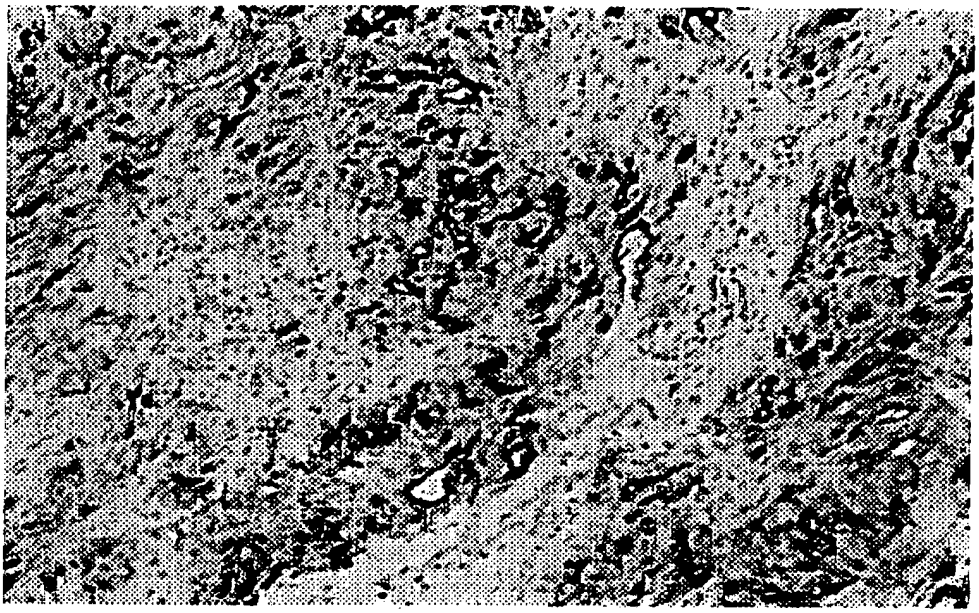

Figure 7. A section from a serous ovarian adenocarcinoma reacted with an antibody against CA-125. The tumor cells show strong labeling of their apical surfaces.

vimentin-positive, but generally fail to express keratin in fixed tissue.[50, 51] Epithelial membrane antigen (EMA) is also negative.[12] The absence of keratin may occasionally be useful in separating a granulosa cell tumor from a poorly differentiated carcinoma. In granulosa-theca cell tumors, the thecal component expresses both keratin and vimentin.[77] In sex cord-stromal tumors, vimentin is expressed, although small amounts of keratin may also be seen.[77] Thecoma-fibroma tumors do not express smooth muscle actin, which may be useful for separating them from the rare ovarian leiomyomas.[14] Leydig cell tumors express vimentin as their only intermediate filament protein.[51]

Diagnosis of Germ Cell Tumors

Although limited studies on ovarian germ cell tumors have been reported, the results show a general concordance with similar tumors in males. Dysgerminomas generally do not express keratin, whereas embryonal carcinomas, endodermal sinus tumors, and choriocarcinomas usually express the keratins of simple epithelia.[50, 51] Human chorionic gonadotropin (hCG) can be identified in trophoblastic cells in many germ cell tumors,[30] although it is usually not of diagnostic significance. PLAP is commonly expressed in germ cell tumors and may be useful in a differential diagnosis; however, it should be remembered that it can also be present in other carcinomas, particularly those of gynecologic origin.[18, 47]

PLACENTA

Separation of Decidual Cells from Trophoblasts

All trophoblastic cells express the keratins of simple epithelium (keratins 8, 18, and 19), whereas decidual cells, despite their epithelial-like appearance, do not.[35] The trophoblastic cells in hydatidiform moles demonstrate the same pattern of keratin expression.[51] Trophoblasts are also positive with human placental lactogen (hPL).[6] Placental site trophoblastic tumors are generally diffusely positive for keratin and hPL, with focal positivity with hCG. These same markers may occasionally be useful in the identification of trophoblasts to rule out an ectopic pregnancy.

Diagnosis of Complete Mole

Human chorionic gonadotropin can be helpful in separating partial mole that expresses hCG moderately early in gestation but shows only weak positivity after 13 to 14 weeks. This contrasts with the strong expression observed in complete moles regardless of gestational age.[6] Another marker useful in separating partial from complete mole is hPL, which is strongly positive in partial mole but only weakly positive in complete mole.[6, 39] PLAP may also be useful, as it becomes increasingly positive with gestational age in partial moles, but is only weakly expressed in complete mole.[6]

Confirmation of Small Cell Carcinoma

Small cell carcinomas with "neuroendocrine" features can occur in the cervix, endometrium, or ovary. In some situations, it may be useful to confirm this morphologic impression by demonstrating positivity with a neuroendocrine marker.[1, 76]

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